Journal of Pharmaceutical and Biomedical Analysis 159 (2018) 179–185

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Study on the compatibility interactions of formula Ding-Zhi-Xiao-Wan based on their main components transport characteristics across Caco-2 monolayers model

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ARTICLE INFO

Article history:
Received 15 April 2018
Received in revised form 25 May 2018
Accepted 30 June 2018
Available online 4 July 2018

Keywords:
Caco-2 cell monolayer
Ding-Zhi-Xiao-Wan
Compatibility interaction
Decompose prescription
Mass spectrometry

ABSTRACT

Influencing the absorption of effective components in the intestines is one of the important compatibility mechanisms of the traditional Chinese medicine. Simulation of drug absorption through an in vitro intestinal epithelial cell line is an important method to study the interaction of drug compatibility and bioavailability of drugs. In this study, the compatibility mechanism of Ding-Zhi-Xiao-Wan (DZXW) was investigated by using the in vitro Caco-2 cell monolayers model. Decomposing the formula into single herb and drug-pair to clarify the compatibility mechanism was firstly used. The transport characteristics of 20 major bioactive compounds including 8 ginsenosides, 6 poria triterpenes, 3 onjisaponins, one polygala oligosaccharide and two essential oils were selected as the main evaluation factor, and the absorption of these compounds by Caco-2 cells in the single herb group, drug-pair group and DZXW group were detected by ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS). The results showed that the absorption of ginsenosides and polysaponins were related to the numbers of glycosyl groups, and the uptaken of poria triterpenes was dominated by lipophilicity. Polygala radix played a critical role in the permeability of gensenosides, and acorus tatarinowii rhizome dominated permeability of poria triterpenes. The apparent permeability coefficients of ginsenosides and poria triterpenes were greater than 14.0×10^{-6} cm s^{-1}, indicating they could be absorbed well, and the ginseng and poria cocos might played the crucial role in the efficacy of DZXW. Herbal combination could remarkable improve the absorption of 18 compounds and the scientific rationality compatibility of DZXW formula was proved.

1. Introduction

Ding-Zhi-Xiao-Wan (DZXW), a famous formula of the Traditional Chinese Medicine (TCM), was firstly recorded in BeijiQianjinYaofang < Thousand-Golden-Prescriptions > by Sun Simiao (581–685 A.D.). It contains four herbs, Ginseng Radix (GR), Poria cocos (P), Polygala Radix (PR), and Acorus Tatarinowii Rhizoma (ATR) in the weight ratio of 3:3:2:2. [1]. It has been used for treating mental disorders in China for thousands of years. In our previous work we had identified 64 components such as triterpenoids, polygala saponins, oligosaccharide esters, sucrose esters, xanthone C-glycosides and ginsenosides [2]. In the formula, GR was "monarch" drug, P was "minister" drug, PR and ATR were "assistant" drug and "messenger" drug, respectively. DZXW can hit multiple targets and produce a synergistic therapeutic effect based on the multi-ingredient preparations being quite different from modern drugs that often focus on a single chemical entity [3], and also exhibited the fewer side effects and relatively low cost. Therefore, DZXW is still utilized to treat the emotional disease clinically till now. For orally administered drugs, intestinal absorption is an important process for the drug action of TCMs. In order to clarify the effective compounds and compatibility mechanism of DZXW formulas drug interactions, it is necessary to understand their intestinal absorption process [4].

The Caco-2 cell monolayers model (human colon adenocarcinoma cell line) has been well recognized as a useful tool to simulate intestinal absorption in vitro [5–8]. It has been used to explain

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https://doi.org/10.1016/j.jpba.2018.06.067
0731-7085/

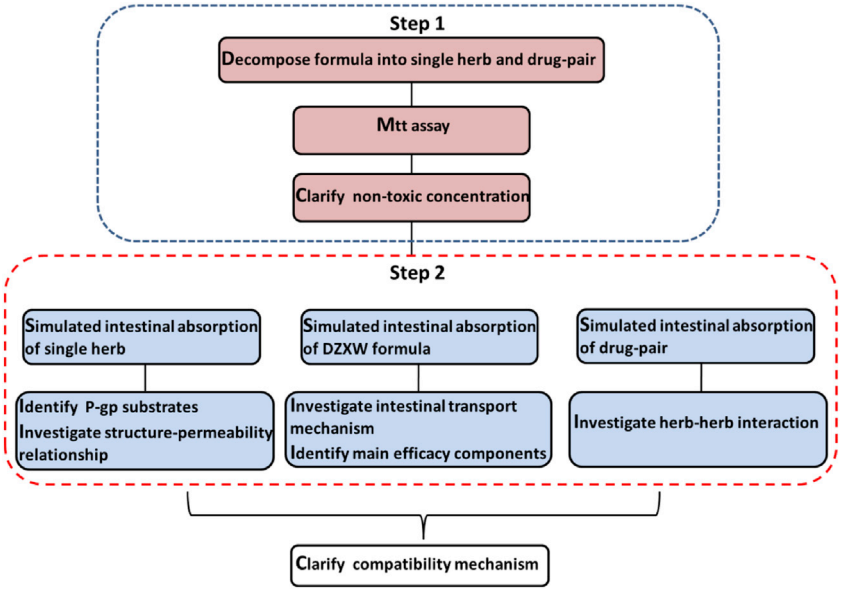

Fig. 1. The systematic workflow of this study.

the transported mechanisms of daily food [9–14], drugs [15–19], nanoparticle drug carrier [20], single herbs [21–23] and the drug-herb interactions [24–27]. In recent years, many researchers focus on the herb-herb interaction [28–30]. Nevertheless, few studies are performed about the impact of herbal compatibility interaction on the absorption of bioactive compounds at the level of intestinal absorption and bioavailability [31,32]. In this study, the intestinal transport process of DZXW was investigated by using caco-2 cell monolayer (Fig. 1), and DZXW formula was decomposed into four single herbs (GR, P, PR, ATR), as well as six herb-pairs (GR + P, GR + PR, GR + ATR, P + PR, P + ATR, PR + ATR). The structure-permeability relationship was studied by determining the Papp values of major bioactive compounds in the single herbs and also the therapeutic ingredients were found out in DZXW. Moreover, the effect of the introduction of each herb on the transport of active ingredients was clarified though the drug-pairs. The scientific rationality compatibility of DZXW formula was also proved.

2. Experiment

2.1. Materials and methods

Poria cocos, Polygala Radix and Acorus Tatarinowii Rhizoma were purchased from Hebei Kaida Traditional Chinese Medicine Co. Ltd (Hebei, China). Ginseng Radix was obtained from Jilin FuSong (Fusong, China), and ginsenosides reference standards were supported by Jilin University (Changchun, China). 6 poria triterpenes, 3 onjisaponins and one polygala oligosaccharide were purchased from PUSH BIO-TECNOLOGY Co. Ltd (Chengdu, China). All herb medicines were identified by Prof. Qinghuang (Changchun Institute of Applied Chemistry, Chinese Academy of Sciences). Deionized water was prepared using a Milli-Q water purification system (Milford, MA, USA). HPLC-grade acetonitrile and formic acid were obtained from Fisher Scientific (Loughborough, UK). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from HyClone Laboratories (GE Healthcare Life Sciences, Uath, USA). Foetal bovine serum (FBS) and 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) were obtained from BI (Biological Industries Israel Beit Haemek LTD., CT, USA). Transwell™ plates of 12 wells (12 mm, pore size 0.4 μm) were purchased from Corning Costar (Cambridge, MA, USA).

2.2. Preparation of the herbal extracts

DZXW extractions were prepared respectively by reflux extracting the four herbs (GR 15 g, P 15 g, PR 10 g, ATR 10 g) in 400 ml of 75% ethanol for three times (2 h). The decoctions were combined, filtered, and then concentrated in vacuum to 50 mL at 60 °C and the final concentration of the obtained crude drug was 1 g/mL. The extraction methods of each single herb (GR, P, PR and ATR) and herb-pairs (GR + P, GR + PR, GR + ATR, P + PR, P + ATR and PR + ATR) were the same as DZXW.

2.3. Cell culture and evaluate the viability of cells

Human colon adenocarcinoma cell line Caco-2 was purchased from Cell Bank of Chinese Academy of Sciences (CBCAS, Shanghai, China). Caco-2 cells were cultured in DMEM, supplemented with 10% (v/v) FBS and 1% penicillin-streptomycin solution at 37 °C in an incubator containing 5% CO_2. The passage number of the cells was 30–45. Viability of cells was directly measured using MTT assay to evaluate the maximum nontoxic concentration to the cells. For the MTT assay, Caco-2 cells were seeded onto a 96-well culture plate at a density of 6000 cells/well and incubated at 37 °C for 24 h. Then the medium was replaced with 100 μL different concentrations of herbal extract solution which diluted in DMEM containing 10% FBS, while the control group was treated with the same amount solvent without drugs. The wells without cells were used as blank control. After 3 h, the medium was replaced with 100 μL MTT at a concentration of 1 mg/ mL. And the MTT solution was removed and the formazan crystals were dissolved in 150 μL DMSO for 4 h. The absorbance at 570 nm was showed on a microplate reader (Tecan, Austria). The percentage of cell viability was calculated as follows:

$$\text{Cellviability\%} = (\text{model-blank})/(\text{control-blank}) \times 100\%$$

2.4. Evaluation of Caco-2 cell monolayers

The formation of functional epithelial layers were monitored by measuring the transepithelial electrical resistance (TEER) with an epithelial volt-ohm meter in order to evaluate the integrity of the Caco-2 cell monolayers before every experiments [33]. Only cell monolayers with a TEER of above 500 Ω/cm^2 were used